

⑫

EUROPEAN PATENT SPECIFICATION

④⑤ Date of publication of patent specification: **02.12.81**

②① Application number: **78300832.9**

②② Date of filing: **15.12.78**

⑤① Int. Cl.³: **A 61 K 31/335,**
C 07 D 493/22,
C 07 D 493/20
/(C07D493/22, 321/00,
311/00, 311/00, 307/00,
493/20, 321/00, 311/00,
311/00)

⑤④ **A milbemycin compound or a mixture thereof for use in the treatment of helminthic infections and anthelmintic compositions containing such compounds.**

③⑩ Priority: **19.12.77 US 861808**

④③ Date of publication of application:
11.07.79 Bulletin 79/14

④⑤ Publication of the grant of the European patent:
02.12.81 Bulletin 81/48

⑥④ Designated Contracting States:
DE FR GB

⑤⑥ References cited:
US - A - 3 950 360

⑦③ Proprietor: **MERCK & CO. INC.**
126, East Lincoln Avenue P.O. Box 2000
Rahway, New Jersey 07065 (US)

⑦② Inventor: **Putter, Irving**
Box 436
R.D. No. 1 Martinsville New Jersey 08836 (US)

⑦④ Representative: **Crampton, Keith John Allen et al,**
D YOUNG & CO 10 Staple Inn
London WC1V 7RD (GB)

EP 0 002 916 B1

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European patent convention).

0 002 916

A milbemycin compound or a mixture thereof for use in the treatment of helminthic infections and anthelmintic compositions containing such compounds

5 The milbemycins are a series of thirteen macrolide antibiotics isolated from the fermentation broth of a strain of *Streptomyces* identified as the B—41—146 strain. Nine of the milbemycin compounds and their preparation are disclosed in U.S. Patent 3,950,360 issued April 13, 1976 to Aoki *et al.* A complete disclosure of all thirteen of the milbemycin antibiotics is found in the Journal of Antibiotics 29 (6) June 1976 pages 73—35 to 76—42 and pages 76—14 and 76—16. The compounds are disclosed as having insecticidal and acaricidal activity.

The present invention is based on the fact that the milbemycins have been found to be very potent anthelmintic agents. The present invention provides such compounds for use in the treatment of helminthic infections.

10 The invention also provides a composition for the treatment of helminthiasis in animals, which composition comprises a milbemycin compound or a mixture of milbemycin compounds and an inert carrier.

The milbemycin compounds as originally disclosed in U.S. Patent 3,950,360 consisted of a series of nine compounds, seven of which had had their structures determined. The compounds were named 15 as B—41 antibiotics and given the designation A₁, A₂, A₃, A₄, B₁, B₂, B₃, C₁ and C₂. Since the filing of the original patent application, an additional four compounds have been isolated from the fermentation broth and the structure of all thirteen compounds has been determined. In addition, the nomenclature has been changed from B—41 to milbemycin and the individual designations changed to α 1 to α 10 and β 1 to β 3 recognizing that there are two basic structural differences among the compounds.

20 The structures of the individual milbemycin compounds and the relationship between the old and new nomenclature is found in the following table:

25

30

35

40

45

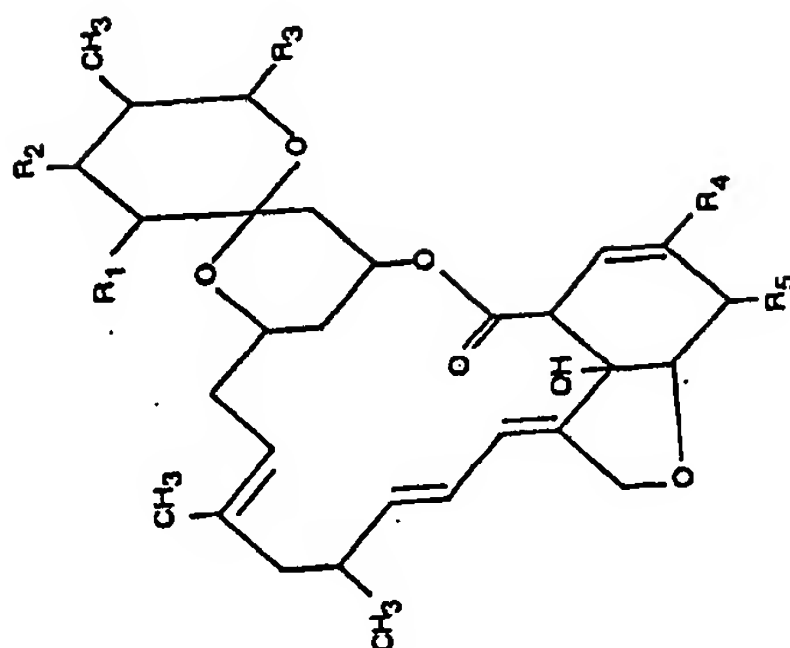
50

55

60

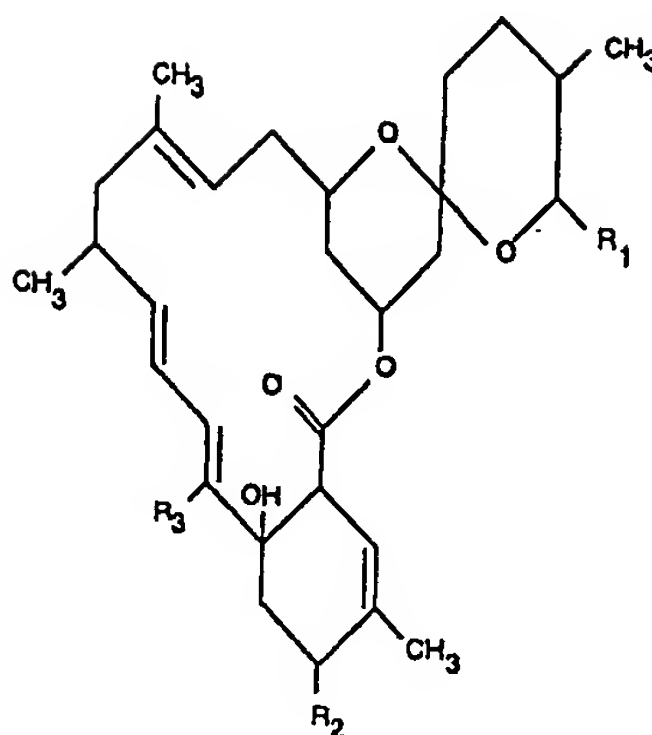
0002916

1	Milbemycin	R ₁	R ₂	R ₃	R ₄	R ₅	B-41
2	α ₁	H	H	CH ₃	CH ₃	—OH	A3
3	α ₂	H	H	CH ₃	CH ₃	—OCH ₃	B2
4	α ₃	H	H	C ₂ H ₅	CH ₃	—OH	A4
5	α ₄	H	H	C ₂ H ₅	CH ₃	—OCH ₃	B3
6	α ₅	—OH	$\begin{array}{c} \text{O} \\ \parallel \\ \text{—OC—CH—C}_4\text{H}_9 \\ \\ \text{CH}_3 \end{array}$	CH ₃	CH ₃	—OH	A2
7	α ₆	—OH	$\begin{array}{c} \text{O} \\ \parallel \\ \text{—OC—CH—C}_4\text{H}_9 \\ \\ \text{CH}_3 \end{array}$	CH ₃	CH ₃	—OCH ₃	B1
8	α ₇	—OH	$\begin{array}{c} \text{O} \\ \parallel \\ \text{—OC—CH—C}_4\text{H}_9 \\ \\ \text{CH}_3 \end{array}$	C ₂ H ₅	CH ₃	—OH	
9	α ₈	—OH	$\begin{array}{c} \text{O} \\ \parallel \\ \text{—OC—CH—C}_4\text{H}_9 \\ \\ \text{CH}_3 \end{array}$	C ₂ H ₅	CH ₃	—OCH ₃	
10	α ₉	H	H	CH ₃	$\begin{array}{c} \text{—CH}_2\text{—O—C—} \\ \parallel \\ \text{O} \end{array}$	$\begin{array}{c} \text{—CH}_2\text{—O—C—} \\ \parallel \\ \text{O} \end{array}$	C1
11	α ₁₀	H	H	C ₂ H ₅	$\begin{array}{c} \text{O} \\ \parallel \\ \text{—CH}_2\text{OC—} \end{array}$	$\begin{array}{c} \text{—CH}_2\text{OC—} \end{array}$	C2



0 002 916

5
10
15



20
25

Milbemycin	R ₁	R ₂	R ₃	B—41
β_1	CH ₃	—OCH ₃	—CH ₂ OH	Al
β_2	C ₂ H ₅	—OCH ₃	—CH ₂ OH	
β_3	CH ₃	—OH	—CH ₃	

The milbemycin compounds are isolated from the fermentation broth by extraction of the mycellia or concentrated filtrate with acetone. The acetone layer is extracted with hexane and concentrated to give a viscous oil. The oil is repeatedly chromatographed on columns of silica gel and alumina, and the columns eluted gradiently with various organic solvent mixtures. Additional chromatographic techniques such as thin layer chromatography and preparative layer chromatography are employed to isolate the individual milbemycin compounds.

The milbemycin compounds of this invention have been found to possess significant parasiticidal activity as anthelmintics in human and animal health.

The disease or group of diseases described generally as helminthiasis is due to infection of an animal host with parasitic worms known as helminths. Helminthiasis is a prevalent and serious economic problem in domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. Among the helminths, the group of worms described as nematodes causes widespread and often times serious infection in various species of animals. The most common genera of nematodes infecting the animals referred to above are *Haemonchus*, *Trichostrongylus*, *Ostertagia*, *Nematodirus*, *Cooperia*, *Ascaris*, *Bunostomum*, *Oesophagostomum*, *Chabertia*, *Trichuris*, *Strongylus*, *Trichonema*, *Dictyocaulus*, *Capillaria*, *Heterakis*, *Toxocara*, *Ascardia*, *Oxyuris*, *Ancylostoma*, *Uncinaria*, *Toxascaris* and *Parascaris*. Certain of these, such as *Nematodirus*, *Cooperia*, and *Oesophagostomum* attack primarily the intestinal tract while others, such as *Haemonchus* and *Ostertagia*, are more prevalent in the stomach while still others such as *Dictyocaulus* are found in the lungs. Still other parasites may be located in other tissues and organs of the body such as the heart and blood vessels, and subcutaneous and lymphatic tissue. The parasitic infections known as helminthiasis lead to anemia, malnutrition, weakness, weight loss, and severe damage to the walls of the intestinal tract and other tissues and organs and, if left untreated, may result in death of the infected host.

The present compounds are also useful against parasites which infect humans. The most common genera of parasites of the gastro-intestinal tract of man are *Ancylostoma*, *Necator*, *Ascaris*, *Strongyloides*, *Trichinella*, *Capillaria*, *Trichuris*, and *Enterobius*. Other medically important genera of parasites which are found in the blood or other tissues and organs outside the gastro-intestinal tract are the filarial worms such as *Wuchereria*, *Brugia*, *Onchocerca* and *Loa*, *Dracunculus* and extra intestinal stages of the intestinal worms *Strongyloides* and *Trichinella*.

These compounds may be administered orally in a unit dosage form such as a capsule, bolus or tablet, or as a liquid drench where used as an anthelmintic in mammals. The drench is normally a solution, suspension or dispersion of the active ingredient usually in water or a suitable non-toxic solvent together with a suspending agent such as bentonite and a wetting agent or like excipient. Generally, the drenches also contain an antifoaming agent. Drench formulations generally contain from 0.01 to 0.50% by weight of the active compound. Preferred drench formulations may contain from 0.01 to 0.1% by weight. The capsules and boluses comprise the active ingredient admixed with a carrier vehicle such as starch, talc, magnesium stearate, or di-calcium phosphate.

Where it is desired to administer the milbemycin compounds in a dry, solid unit dosage form,

capsules, boluses or tablets containing the desired amount of active compound usually are employed. These dosage forms are prepared by intimately and uniformly mixing the active ingredient with suitable finely divided diluents, fillers, disintegrating agents and/or binders such as starch, lactose, talc, magnesium stearate, and vegetable gums. Such unit dosage formulations may be varied widely with respect to their total weight and content of the anthelmintic agent depending upon factors such as the type of host animal to be treated, the severity and type of infection and the weight of the host.

When the active compound is to be administered via an animal feedstuff, it is intimately dispersed in the feed or used as a top dressing or in the form of pellets which may then be added to the finished feed or optionally fed separately. Alternatively, the anthelmintic milbemycin compounds may be administered to animals parenterally, for example, by intramuscular, intratracheal, or subcutaneous injection in which event the active ingredient is dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the active material is suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety such as peanut oil, or cotton seed oil. Other parenteral vehicles such as solketal, glycerol-formal and aqueous parenteral formulations are also used. The active milbemycin compound or compounds are dissolved or suspended in the parenteral for administration; such formulations generally contain from 0.05 to 50% by weight of the active compound.

Milbemycin compounds may also be administered topically by admixture in a suitable vehicle such as dimethylsulfoxide or a hydrocarbon solvent. This preparation is then applied directly to the external surface of the animal by techniques such as spraying or direct pouring.

The optimum amount of active compound to be employed for best results will, of course, depend upon the particular compound employed, the species of animal to be treated and the type and severity of helminthic infection or infestation. Generally, good results are obtained by the oral administration of one or more of the milbemycin compounds at a rate of from 0.01 to 100.0 mg. per kg. of animal body weight, such total dose being given at one time or in divided doses over a relatively short period of time such as 1—5 days. With the preferred compounds of the invention, excellent control of such parasites is obtained in animals by administering from 0.5 to 50.0 mg. per kg. of body weight in a single dose. Repeat treatments are given as required to combat re-infections and are dependent upon the species of parasite and the husbandry techniques being employed. The techniques for administering these materials to animals are known to those skilled in the veterinary field.

When the compounds described herein are administered as a component of the feed of the animals, or dissolved or suspended in the drinking water, compositions are provided in which the active compound or compounds are intimately dispersed in an inert carrier or diluent. By inert carrier is meant one that will not react with the anthelmintic agent and one that may be administered safely to animals. Preferably, a carrier for feed administration is one that is, or may be, an ingredient of the animal ration.

Suitable compositions include feed premixes or supplements in which the active ingredient is present in relatively large amounts and which are suitable for direct feeding to the animal or for addition to the feed either directly or after an intermediate dilution or blending step. Typical carriers or diluents suitable for such compositions include, for example, distillers' dried grains, corn meal, citrus meal, fermentation residues, ground oyster shells, wheat shorts, molasses solubles, corn cob meal, edible bean mill feed, soya grits, and crushed limestone. The active compounds are intimately dispersed throughout the carrier by methods such as grinding, stirring, milling or tumbling. Compositions containing from 0.05 to 20.0% by weight of the active compound are particularly suitable as feed premixes. Feed supplements, which are fed directly to the animal, contain from 0.002 to 0.30% by weight of the active compounds.

Such supplements are added to the animal feed in an amount to give the finished feed the concentration of active compound desired for the treatment and control of parasitic diseases. Although the desired concentration of active compound will vary depending upon the factors previously mentioned as well as upon the particular milbemycin compound or combination of milbemycin compounds employed, the compounds of this invention are usually fed at concentrations of 0.0001 to 0.02% in the feed in order to achieve the desired antiparasitic result.

In using the compounds of this invention, the individual milbemycin components may be isolated and purified and used in that form. Alternatively, mixtures of two or more of the individual milbemycin components may be used. It is not necessary to completely separate the various milbemycin compounds obtained from the purification of the fermentation broth. Generally, there is obtained a mixture containing two or more of the milbemycin compounds, but having other unrelated compounds excluded therefrom, and such mixture may be used for the prevention and treatment of helminthic diseases as described herein. Such a mixture generally will contain unequal proportions of the milbemycin compounds, however, all of the compounds have substantial activity and the antiparasitic activity of the mixture can be accurately determined.

The compounds of this invention have a broad spectrum of activity against many internal parasites at low dosage levels and in many different animals. At levels of about 2.5 mg. per kg. of animal body weight, concentrated mixtures of milbemycin compounds may be administered to sheep infected with *Haemonchus contortus*, *Ostertagia circumcincta*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Cooperia spp.*, and *Oesophagostomum columbianum*. Similarly in cattle at dosages as

low as 2.5 mg./kg. the milbemycin compounds may be used against *Ostertagia ostertagi*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Oesophagostomum radiatum* and *Dictyocaulus viviparus*. (In addition, horses infected with bots (*Gastrophilus intestinalis* and *Gastrophilus haemorrhoidalis*), large and small strongyles and *Oxyuris* may be treated with milbemycin.) In rodents, such as mice, infections of *Syphacia*, *Nematospiroides* and *Aspicularis* may be treated by the oral administration of the milbemycin compounds or of the concentrates obtained from the extraction of the mycelia.

The anthelmintic activity of the milbemycin compounds may be determined by orally administering *via* the feed, a sample of milbemycin individual compound, a mixture of milbemycin compounds, or a concentrated extract, to mice which had been infected 3 days earlier with *Nematospiroides dubius*. At 11, 12 and 13 days after the initiation of the medication, the faeces of the mice are examined for *N. dubius* eggs, and on the next day the mice are sacrificed and the number of worms present in the proximal portion of the small intestine are determined. An active compound is observed when there is a significant reduction of egg and worm counts when compared to infected unmedicated controls.

Claims

1. A milbemycin compound or a mixture of milbemycin compounds for use in the treatment of helminthic infections.
2. A composition for the treatment of helminthiasis in animals, which composition comprises a milbemycin compound or a mixture of milbemycin compounds and an inert carrier.
3. An orally administrable composition as claimed in Claim 2.
4. A composition as claimed in Claim 3 in which the inert carrier is an element of the animals' feed.
5. A composition as claimed in Claim 4 in the form of a feed supplement containing from 0.002 to 0.30% by weight of the milbemycin compound or compounds.
6. A composition as claimed in Claim 4 in the form of a feed premix containing from 0.05 to 20.0% by weight of the milbemycin compound or compounds.
7. A composition as claimed in Claim 4 in the form of a finished feed product containing from 0.0001 to 0.02% by weight of the milbemycin compound or compounds.
8. A parenterally administrable composition as claimed in Claim 2.
9. A topically administrable composition as claimed in Claim 2.
10. A composition as claimed in Claim 9 in which the milbemycin compound or compounds is or are admixed with dimethylsulfoxide or a hydrocarbon solvent.

Revendications

1. Une milbemycine ou un mélange de milbemycines pour l'utilisation dans le traitement des infections helminthiques.
2. Une composition pour le traitement des helminthiasis chez les animaux, composition qui comprend une milbemycine ou un mélange de milbemycines et un véhicule inerte.
3. Une composition administrable par voie orale selon la revendication 2.
4. Une composition selon la revendication 3, dans laquelle le véhicule inerte est un élément de l'alimentation de l'animal.
5. Une composition selon la revendication 4, sous la forme d'un supplément alimentaire contenant de 0,002 à 0,30% en poids d'une ou plusieurs milbemycines.
6. Une composition selon la revendication 4, sous la forme d'un pré-mélange alimentaire contenant de 0,05 à 20,0% en poids d'une ou plusieurs milbemycines.
7. Une composition selon la revendication 4, sous la forme d'un produit alimentaire fini contenant de 0,0001 à 0,02% en poids d'une ou plusieurs milbemycines.
8. Une composition administrable par voie parentérale selon la revendication 2.
9. Une composition pour l'usage topique selon la revendication 2.
10. Une composition selon la revendication 9, dans laquelle la ou les milbemycines est ou sont mélangées avec du diméthylsulfoxyde ou un solvant hydrocarboné.

Patentansprüche

1. Milbemycine oder Gemische von Milbemycinen zur Behandlung von Wurmbefall.
2. Mittel zur Behandlung von Helminthose bei Tieren, enthaltend ein Milbemycin oder ein Gemisch von Milbemycinen und einen inerten Trägerstoff.
3. Oral verabreichbares Mittel nach Anspruch 2.
4. Mittel nach Anspruch 3, bei dem der inerte Trägerstoff ein Bestandteil des Tierfutters ist.
5. Mittel nach Anspruch 4 in Form eines Futtermittelzusatzes, enthaltend 0,002 bis 0,30 Gewichtsprozent an Milbemycin oder Milbemycinen.

0002916

6. Mittel nach Anspruch 4 in Form eines Futtermittelvorgemisches, enthaltend 0,05 bis 20,0 Gewichtsprozent an Milbemycin oder Milbemycinen.

7. Mittel nach Anspruch 4 in Form eines fertigen Futtermittels, enthaltend 0,0001 bis 0,02 Gewichtsprozent an Milbemycin oder Milbemycinen.

5 8. Parenteral verabreichbares Mittel nach Anspruch 2.

9. Topisch verabreichbares Mittel nach Anspruch 2.

10. Mittel nach Anspruch 9, bei dem das Milbemycin oder die Milbemycine mit Dimethylsulfoxid oder einem Kohlenwasserstofflösungsmittel vermischt sind.

10

15

20

25

30

35

40

45

50

55

60

65